SYNAPTIC TRANSMISSION

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Nerve cells communicate with each other through specialized regions of contact called synapses. There are estimated to be about 4×10^{11} such contacts per gram of guinea-pig cerebral cortex;[1] this suggests that the human brain may contain as many as 10^{14} contacts, a level of connectivity truly remarkable in both its complexity and its degree of miniaturization. Contacts morphologically similar to synapses occur between nerve and muscle or secretory cells, and since much of our knowledge of synaptic transmission has been obtained with the latter types of contact, it is convenient to consider all of them together.

Types of Synapse.—Chemical synapses: The contact which a motor nerve makes with striated muscle, the neuromuscular junction (Fig. 1), is perhaps the synapse—in this broader sense—about which most is known. The axon of the motor neuron undergoes an expansion at its ending to form a flattened bag, the under surface of which is closely applied to, but does not touch, the postjunctional membrane. The cleft between the pre- and postjunctional membranes is enlarged by the existence of folds in the latter; its function seems to be to prevent the excitation of the postjunctional element by the electrotonic spread of current from the presynaptic nerve when impulses traveling down the axon reach the terminal. Transmission of excitation across the gap is achieved instead by the release of a specific chemical transmitter substance, the identity of which is known in this case: it is acetylcholine. If acetylcholine is applied in minute amounts to a junction through a micropipette, it generates a graded postjunctional potential similar to that seen during synaptic transmission; the synaptic and evoked potentials respond similarly to the presence of drugs.[2]

Some years ago, the important observation was made[3] that in the resting junction there were small, transient postjunctional potentials occurring randomly in time, similar to the full-scale potentials that signalize transmission, but too small to generate a contraction. The known electrogenic properties of acetylcholine showed that these miniature potentials could not be caused by the resting diffusion of acetylcholine out of the presynaptic nerve terminal molecule by molecule, but rather that "packets" or quanta of acetylcholine variously estimated to contain between 1,000 and 10,000 molecules were being released in random fashion. The full-scale potential recorded during transmission was shown to be due to the synchronized release of a large number of these quanta over a very short time interval. Similar "minipotentials" have been detected at central synapses,[4] and it is thought that transmitter release is quantized there too.

About the time that minipotentials were discovered, the first high-resolution electron micrographs of synapses were being made.[5–8] These showed that a highly characteristic feature of the presynaptic terminal cytoplasm was the presence of large numbers of small vesicles about 500 Å in diameter (Fig. 1*B*). It was natural to speculate that these might be the morphological counterparts

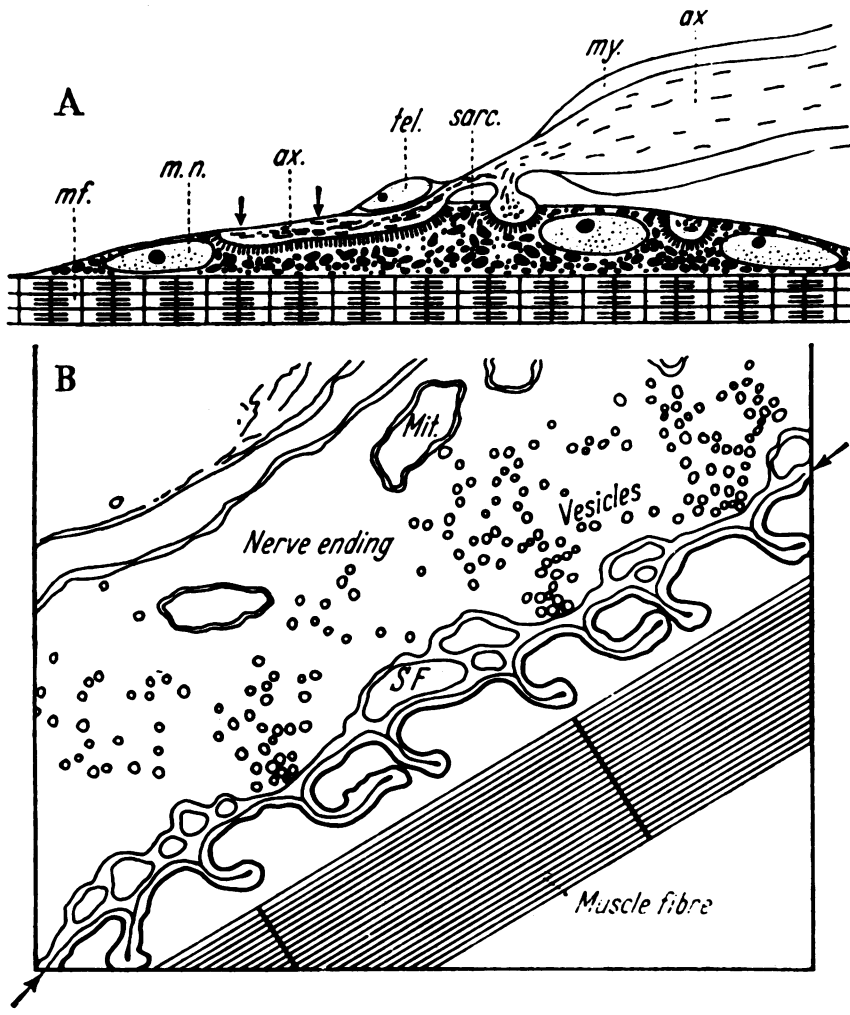

FIG. 1.—(*A*) Diagram of a motor endplate showing axoplasm (*ax.*) and myelin (*my.*) of motor nerve, and saclike terminals (*arrows*) lying in gutterlike depressions of the mitochondrion-rich muscle sacroplasm (*sarc.*). The terminals are protected by teloglia (*tel*), and muscle nuclei (*m.n.*) and myofibrils (*mf.*) are also diagrammed.

(*B*) Tracing of an electron micrograph of a portion of the nerve terminal similar to that between the arrows in (*A*). Note the highly folded postsynaptic membrane extending into the muscle sacroplasm, the fingerlike projections of teloglia (*SF*), and the numerous vesicles and mitochondria (*Mit.*) in the terminal cytoplasm.

of the quanta. Structures of this size could contain the requisite amounts of acetylcholine. It has since proved possible to isolate very pure preparations of synaptic vesicles and to demonstrate directly that they contain acetylcholine in roughly the right amount.[9]

In the central nervous system (Fig. 2) the terminal axons and their presynaptic swellings are much smaller than at the neuromuscular junction, and considerable

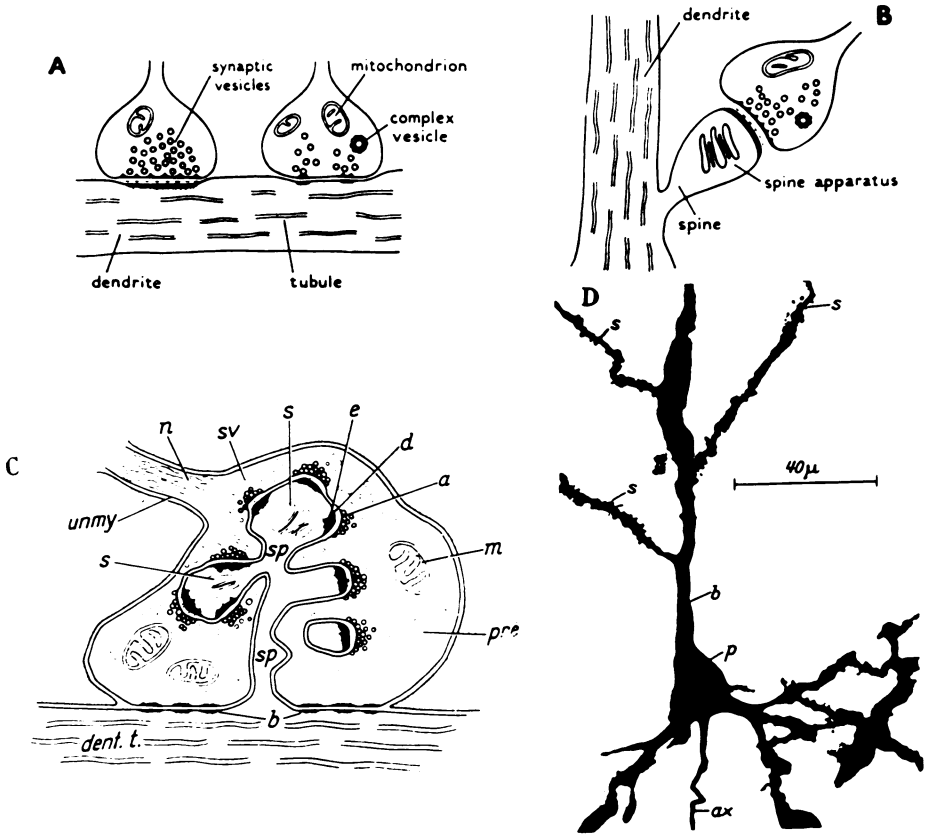

FIG. 2.—Types of central synapse. (*A*) Type 1 and type 2 synapses on a dendrite. (*B, C*) The attachment is with a dendrite spine (*sp*) which, in hippocampal pyramidal cells (*C*) may invaginate the presynaptic ending (*pre*). Synaptic vesicles (*sv*) often form clusters (*a*) opposite thickenings (*d, e*) on the spine, which is seen to contain an array of flattened vesicles and membranes (*s*). The unmyelinated preterminal axon (*unmy*) contains neurofilaments (*n*); the dendrite, anchored to the presynaptic nerve terminal by symmetrical attachment plaques (*b*), around which there is no special concentration of vesicles, is filled with neurotubules (*dent. t.*). (*D*) shows a Golgi preparation of a cortical neuron in which the perikaryon (*p*), dendrites (*b*) with spines (*s*), and axon (*ax*) are visible.

morphological variations occur. However, in most vertebrate synapses the clearly defined gap between pre- and postsynaptic membranes and the presence of numerous 500-Å vesicles in the presynaptic terminal cytoplasm are constant features which lead us to believe that such synapses utilize chemical transmitters also, though not necessarily acetylcholine.

Besides the 500-Å vesicles, four other types—larger vesicles about 1000 Å in diameter, elongated vesicles,[10] dense-core vesicles, and "coated" vesicles—occur in certain types of terminal and in terminals fixed in certain ways. These differences in vesicle morphology may reflect differences in the type of transmitter utilized; thus there is good evidence that dense cores are associated with the monoamine transmitters noradrenaline and 5-hydroxytryptamine,[11] and some

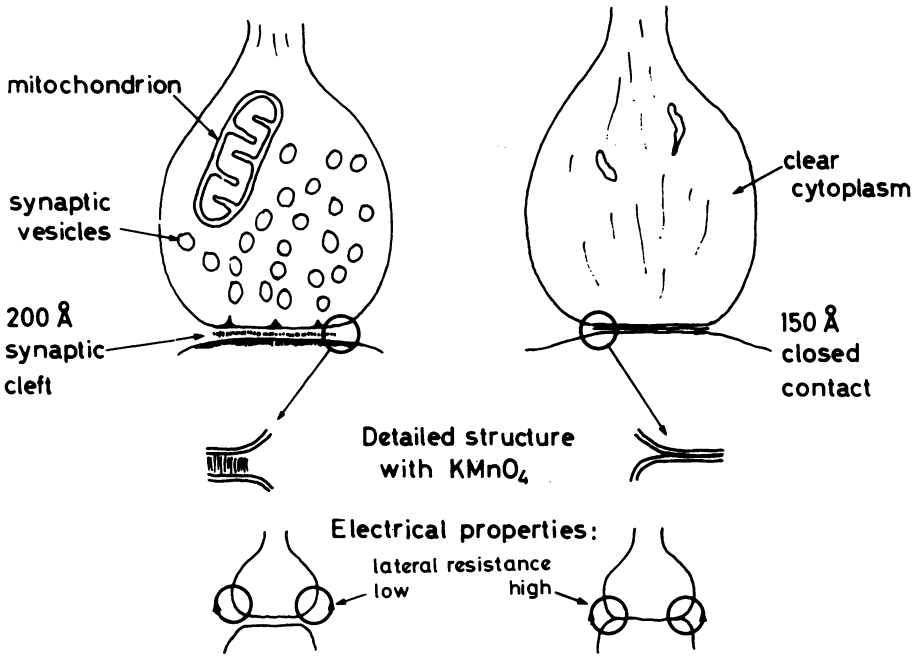

FIG. 3.—Characteristics of open, chemical synapse (*left*) and closed, electrical synapse (*right*). Note in the latter synapse the absence of synaptic vesicles and cleft and the invasion of the postsynaptic elements by action currents which in the chemical synapse are short-circuited by the low-resistance cleft.

reason for thinking that elongated vesicles (observed most clearly after formaldehyde fixation) may be characteristic of synapses exerting an inhibitory effect on the postsynaptic cell.[12] The presumed chemical transmitter at such synapses brings about a hyperpolarization, that is, an increase in the membrane potential of the postsynaptic cell (probably by increasing the permeability of the membrane to chloride ions), thus making it more difficult to depolarize by excitatory transmitters.[13]

Electrical synapses: The identification of synapses as chemical on the basis of their morphology has been strengthened by the discovery[14] of true electrical synapses, that is, synapses in which transmission occurs as the result of the electrotonic spread of current from the pre- to the postsynaptic cell. Such synapses are characterized morphologically[15] (Fig. 3) by presynaptic swellings filled with a watery cytoplasm from which vesicles are absent and by close contact, without any intervening 200-Å cleft, between the pre- and postsynaptic membranes. Mixed types of synapse are also known: significantly, the vesicles in such synapses occur in the neighborhood of the 200-Å cleft; the cytoplasm adjacent to regions of close contact is free of vesicles.

The Identity of Central Transmitters.—Acetylcholine: Besides being the transmitter at the neuromuscular junction, acetylcholine is also the transmitter at autonomic ganglia, at certain postganglionic autonomic endings, and at very

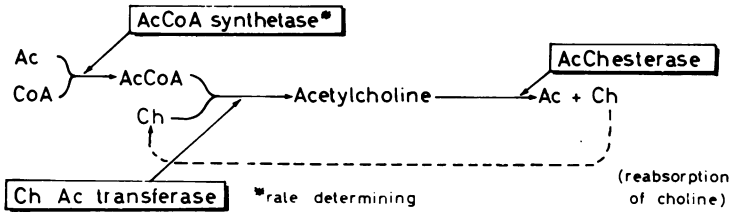

FIG. 4.—Synthesis and destruction of acetylcholine by nervous tissue. (*Ac*, acetate or acetyl; *Ch*, choline.)

many central synapses. It is synthesized in nervous tissue (Fig. 4) by the enzyme choline acetyltransferase from acetyl-coenzyme A and choline, and rapidly hydrolyzed after release to acetate and choline by the enzyme acetylcholinesterase. Since the products have less than one ten thousandth of the transmitter activity of acetylcholine, this hydrolysis very effectively terminates the transmitter action. Degeneration studies show that all three components—choline acetyltransferase, acetylcholine, and acetylcholinesterase—are constituents of cholinergic neurons and, although concentrated in the nerve endings, are distributed in smaller amounts throughout the length of the neuron. However, there is evidence that acetylcholinesterase is functionally on the outside of the neuron and only comes into contact with acetylcholine after the latter has been released. Acetylcholinesterase hydrolyzes acetylthiocholine as well as acetylcholine, and in the presence of copper the thiocholine released is precipitated. This may be used as the basis of a histochemical method for cholinesterase, in which the copper thiocholine is converted to a black deposit of copper sulfide.

With the use of this method, it has been shown that many of the cholinergic neurons of the central nervous system form a great ascending system of neurons originating from cell bodies in the reticular formation and radiating to all parts of the forebrain: to the hypothalamus, thalamus, visual pathway, striatum, hippocampus, and neocortex.[16] This system appears to be identical with the ascending reticular activating system, which is responsible for electrocortical arousal and which may well be involved in consciousness and the wakened state.

The monoamines: Noradrenaline, dopamine, and 5-hydroxytryptamine make up a group of related monoamines to which a central transmitter role has been ascribed. Unlike acetylcholine, these amines can be directly visualized histochemically by taking advantage of the green or yellow fluorescent compounds that they form with dry formaldehyde gas. This method has been brilliantly developed and used by a large group of Swedish workers to trace peripheral and central monoaminergic neurons. Once again, the transmitter is found to be highly concentrated in the nerve terminals, but by special methods cell bodies and axons can also be rendered fluorescent. Such studies show that central neurons containing these transmitters are organized into fairly well defined tracts.[17] The ascending tracts are less extensive than, and distinct from, the cholinergic tracts, but like the latter, they start from the midbrain and innervate many of the same forebrain regions.

Amino acids: There remain many central synapses not accounted for by cholinergic and monoaminergic neurons. There is increasing evidence that certain amino acids, particularly glutamate, γ-aminobutyrate, and glycine may have a transmitter role. Many central synapses are concerned not with excitation but with inhibition. Recent work, using iontophoretic application and intracellular recording from single units by means of micropipettes, shows that γ-aminobutyric acid in the cerebral cortex[18] and glycine in the spinal cord[19] have all the requisite properties for inhibitory transmitters in those areas. Another amino acid, glutamate, has the property of firing most nerve cells, but the changes in the electrical properties of the cell membranes that it produces do not exactly parallel those produced by synaptic stimulation. Both glutamate and γ-aminobutyrate are effective in extremely small amounts; about 10^{-15} of a mole will cause a detectable effect on a single cell. These amino acids, with certain others, exist in nervous tissue in uniquely high concentration; and γ-aminobutyric acid and the enzyme glutamate decarboxylase, which forms it from glutamate, do not occur in other mammalian tissues.

Many drugs that affect the central nervous system are believed to do so by interfering in one way or another with chemical transmission, and some of the most powerful are structural chemical analogues of putative transmitters. In Figure 5 the chemical relationship between certain transmitters and some well-known hallucinogens and tranquilizers is indicated. Significantly, the hallucinogenic agents are chemical analogues of either acetylcholine or the monoamines, substances which, as we have seen, are transmitters in ascending systems possibly concerned with consciousness and perception.

We can only surmise why the evolving nervous system turned to chemical transmission in order to achieve communication between nerve cells. Storage of transmitters in presynaptic nerve terminals may be a device to ensure unidirectionality of transmission: a property of the synapse but not of the axon, and probably essential for the orderly processing of information in the central nervous system. Antidromic impulses traveling up axons into cell bodies are unable to travel further, because the postsynaptic cell does not contain the transmitter whose release is essential for communication across the gap.

Another reason for chemical transmission may be to reduce unwanted "cross-talk" between intertwined but functionally distinct systems. Iontophoretic application of putative transmitters to single neurons of the nervous system shows how chemically specific nerve cells are. A single unit in the cat cortex may be excited by low doses of glutamate, but not at all by acetylcholine. If the tip of the pipette is moved only a few microns deeper, contact may be made with a cell that is responsive to both compounds.

The Isolation of Presynaptic Nerve Terminals and Their Component Organelles.—Synaptosomes: When brain tissue is homogenized in iso-osmotic media (e.g., 0.32 *M* sucrose) under conditions of relatively mild shear, most of the acetylcholine of the original brain tissue remains bound to particulate material, in which state it is both immune to the action of cholinesterase and pharmacological inactive. It may readily be released by procedures that disrupt lipoprotein membranes. The particles bearing the bound acetylcholine may be separated

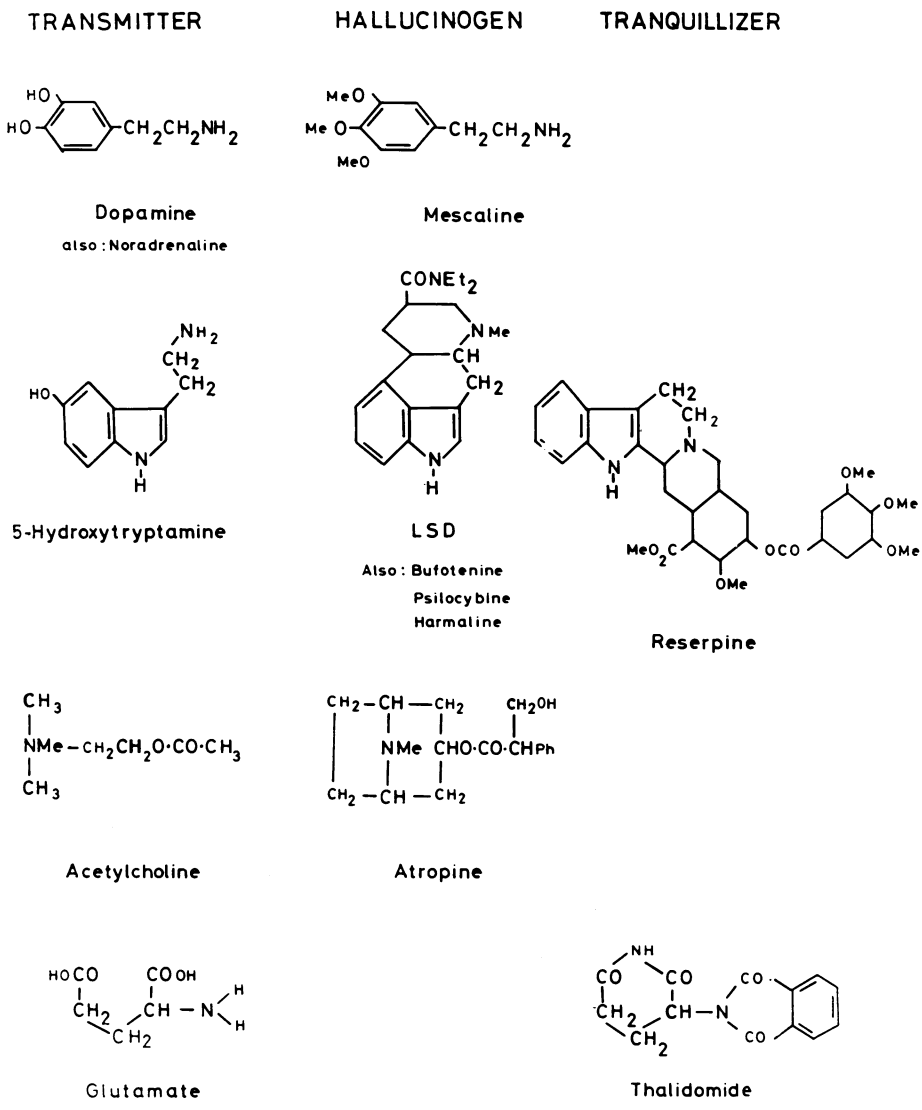

FIG. 5.—Structural relationship between central transmitters and psychoactive drugs.

from nuclei, glial and neuronal fragments, mitochondria, microsomes, and other products of homogenization by differential and density-gradient centrifuging.[20] The fraction so obtained is also rich in other putative transmitters such as noradrenaline and 5-hydroxytryptamine.[21] The significance of these findings became clearer when it was discovered that the fraction consists of an enriched preparation of detached presynaptic nerve terminals[22, 23] to which the name "synaptosomes" has been given.[24] Apparently the physical properties of the presynaptic terminals are such that they survive the general cellular disruption that accompanies homogenization, and "pinch off" to form detached, sealed

structures that retain both the morphology and the transmitter content of the original endings (Fig. 6).

The successful isolation of presynaptic nerve terminals has provided a new type of *in vitro* preparation with which to study the molecular processes involved in the synthesis, storage, release, and ultimate inactivation of transmitter substances,[25] and the effects of drugs on these processes. Other problems that may be studied with synaptosome preparations include the dynamics of axonal flow[26] and the intraterminal synthesis of protein,[27] which may be of considerable importance in relation to the problem of the plasticity of synaptic connections and the mechanisms of memory and learning. The preparation should also serve as a source of new transmitters, provided suitable means can be devised for their detection.[28]

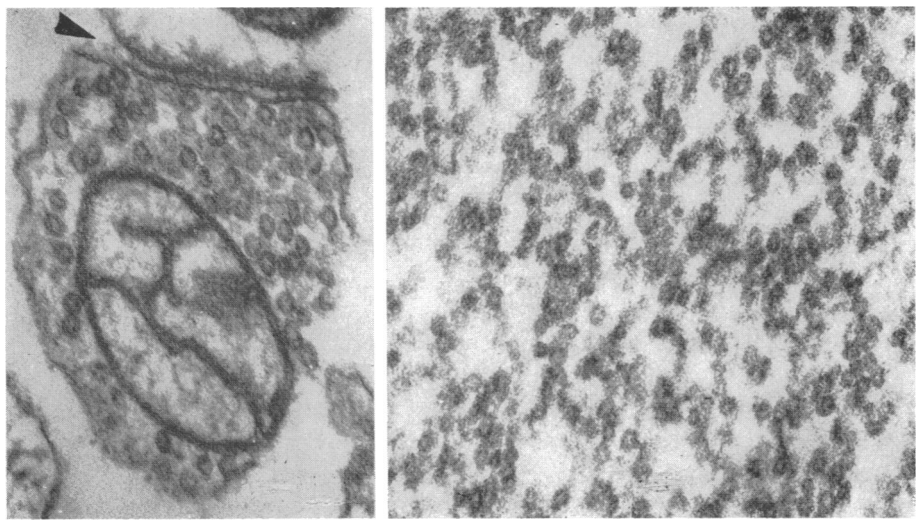

FIG. 6.—Electron micrographs of (*left*) synaptosome (×85,000) showing external membrane, cytoplasm containing synaptic vesicles and a small mitochondrion, and (*arrow*) adherent length of postsynaptic membrane; and (*right*) isolated synaptic vesicles (×70,000).

Recent work with gel filtration through Sephadex[29] in the author's laboratory has confirmed earlier impressions that synaptosomes are sealed structures and contain constituents of soluble cytoplasm, including glycolytic enzymes. Though rather labile, they respire well if incubated with substrates (especially an equimolar mixture of glucose and succinate) and cofactors at 25–30°C, and this respiration is coupled to the synthesis of high-energy phosphate compounds. Actively metabolizing synaptosomes take up choline by a Na^+-dependent, hemicholinium-sensitive mechanism similar to that found in brain slices.[30] The permeability of the external membrane to ions is similar to that of mammalian C-fibers. Thus, in many of their properties, synaptosomes behave as miniature cells and can be used as model neurons.

Synaptic vesicles and external synaptosome membranes: Synaptosomes are osmotically sensitive, and on suspension in water swell up and burst, releasing a

proportion of their cytoplasm, synaptic vesicles (Fig. 6), and intraterminal mitochondria. Density gradient centrifuging permits the separation of these components from each other and from the external membranes of the synaptosomes.[24]

Such preparations enable the chemical organization of the presynaptic terminal to be studied.[31] Acetylcholine exists within the synaptosome in two fractions, a cytoplasmic and a vesicular; it has been estimated that there are about 2000 molecules of acetylcholine per cholinergic vesicle, which is within the lower range of estimates of the size of a quantum of acetylcholine.[9] The two fractions are quite distinct, as has been shown in labeling experiments,[32] the vesicular fraction being negligibly labeled under conditions that result in considerable cytoplasmic labeling. Even in whole brain, the vesicular fraction is more slowly labeled than the cytoplasmic when tritiated choline is the precursor.[33]

By contrast, at the ionic strength prevalent in intact synaptosomes, the enzyme synthesizing acetylcholine, choline acetyltransferase, is all in the soluble cytoplasmic compartment of the synaptosome.[34] The mechanism whereby vesicular acetylcholine is synthesized remains obscure.

The enzyme and lipid composition of vesicles and external membranes has been compared and found to differ in several ways.[35] External membranes contain Na^+, K^+-activated adenosine triphosphatase, cholinesterase, and ganglioside in considerable concentrations; these components are not present in vesicles. External membranes, like myelin and the plasma membranes of other cells, such as liver cells, have a cholesterol:phospholipid molar ratio close to unity; synaptic vesicles have a ratio of about 0.4, similar to microsomes, which are mainly derived from internal membranes.

These differences in composition are relevant to the still unsolved problem of how the transmitter is released from the terminal. On the assumption that release involves the vesicular fraction as the quantal hypothesis requires, it seems unlikely that the vesicles simply fuse with the external wall, but rather likely that they form a membrane system distinct from, but communicating through, the external presynaptic membrane. There might well be an intercommunicating system of fine tubules—hard to see by normal histological methods—composed of protein or another macromolecule that could open or close in response to ionic changes induced by action potentials. In negative staining, fine interconnections between vesicles, as well as detached and exceedingly fine fibrillar material,[35] are sometimes seen.

Postsynaptic membranes: Synaptosomes are not infrequently seen with portions of postsynaptic membranes adhering to them[23, 9] (Fig. 6). The synaptosome preparation is thus a useful potential source of postsynaptic membranes and of the material responsible for the adhesion of the pre- and postsynaptic membranes, a knowledge of which may well be important for our understanding of how specific contacts are made in the developing nervous system.

Summary.—Electron-microscopic, histochemical, and iontophoretic techniques support the concept that transmission at most synapses is brought about by the release of small amounts of chemical transmitter substances. There is now much circumstantial evidence that acetylcholine and noradrenaline, long established as

transmitters in the peripheral nervous system, are central transmitters also. Other central synapses, excitatory and inhibitory, may utilize certain amino acids; among the most potent pharmacologically when applied to single cells are glutamate, aspartate, γ-aminobutyrate, and glycine.

There is also evidence that certain major systems of neurons utilize particular transmitters. Thus acetylcholine and the monoamines appear to be involved in ascending systems concerned in some way with the conscious state; by contrast, γ-aminobutyrate and glycine are the putative transmitters of inhibitory cells in the cortex and spinal cord, respectively.

Chemical transmission may thus be thought of as a kind of chemical coding and may have developed as a device for elimination of unwanted "cross-talk" between functionally distinct systems of neurons. The localization of the transmitter in the presynaptic nerve terminal is also a guarantee of unidirectionality of synaptic transmission—a prerequisite for the orderly relaying of information in the central nervous system.

Many drugs that act on the central nervous system are structural analogues of chemical transmitters, and a useful hypothesis is that they act by interfering at one point or another with the molecular mechanisms involved in the synthesis, storage, release, and ultimate destruction of central transmitters. Certain hallucinogens, e.g., mescaline, LSD, and atropine, are structural analogues of the transmitters (noradrenaline, 5-hydroxytryptamine, acetylcholine) believed to be utilized in the neuron systems involved in consciousness and affective states. The possibility is also being actively discussed that certain psychoses may result from the impaired or abnormal metabolism of some of this group of transmitters.

In recent years, a new approach to the problems of the mechanisms of transmitter metabolism, storage, and release has been made possible by the discovery that presynaptic nerve terminals can be isolated from nervous tissue. If the tissue is dispersed under appropriate conditions in isotonic sucrose, the nerve terminals are pinched off and torn away from their attachments to form sealed structures termed synaptosomes. Centrifugal isolation techniques permit the synaptosomes to be separated from other tissue elements.

Synaptosomes retain both the fine structure and the transmitter content of the original terminals. From them may be prepared samples of terminal cell sap, external membranes, intraterminal mitochondria, and synaptic vesicles. These last are the characteristic organelles (about 500 Å in diameter) of the presynaptic nerve terminals. A study of these organelles is permitting conclusions to be drawn regarding the molecular basis for the storage and release of transmitter substances and the mode of action of drugs and toxins on these processes.

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